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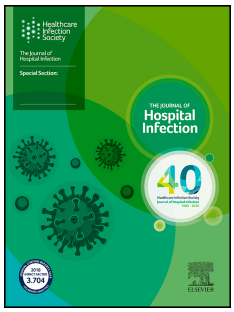

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Successful Management of a *Clostridioides difficile* Ribotype 027 Outbreak with a Lean Intervention Bundle

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Abstract

Background: In a 2015 point prevalence study, *Clostridioides difficile* 027, a hypervirulent ribotype, was absent from healthcare institutions in Switzerland. In late 2016, we detected an outbreak of *C. difficile* infection (CDI) with ribotype 027 occurring across several hospitals in the same hospital network.

Methods: The first cases of CDI due to ribotype 027 triggered an outbreak investigation, including whole genome sequencing (WGS) to identify outbreak strains.

Findings: We identified 28 patients with CDI caused by ribotype 027 between December 2016 and December 2017, out of which twenty were caused by a single clone. Commonalities among these patients were hospitalization in the same room or on the same ward, receiving care from the same healthcare workers, and shared toilet areas. In addition to the epidemiological links suggesting possible transmission pathways between cases, WGS confirmed the clonality of this *C. difficile* 027 outbreak. The outbreak was contained by isolation precautions, raising awareness among healthcare workers, harmonizing diagnostic algorithms, and switching to a sporicidal agent for environmental disinfection. Of note, neither default gowning and gloving nor handwashing with water and soap were implemented.

Conclusions: This *C. difficile* 027 outbreak was recognized belatedly due to lack of screening for this ribotype in some hospitals, and was contained by a swift response with simple infection prevention measures and adapting the laboratory approach. In order to have a better understanding of *C. difficile* epidemiology, diagnostic approaches should be standardized, CDI declared notifiable, and longitudinal data on prevalent ribotypes collected in countries where this is not established.

Introduction

Clostridioides difficile infection (CDI) is a common healthcare-associated infection and often causes outbreaks. These outbreaks can be difficult to manage because transmission not only occurs via contact but also through the environment, where *C. difficile* spores may survive for extended periods of time [1]. Certain ribotypes of *C. difficile* have been found to be more virulent and more likely to sporulate than others. The ribotype 027/NAP1/B1 is considered the most prominent hypervirulent ribotype [2]. It first came to attention in 2000 when an outbreak with unusually poor clinical outcomes was reported from Philadelphia [3]. Since then, *C. difficile* 027 has caused numerous outbreaks in healthcare settings around the world and is feared both for its effect on mortality and the increased risk of recurrent CDI in affected patients [4]. Accordingly, the knowledge on how to best prevent CDI cases and outbreaks has been assembled in practice guidelines such as in the HAI compendium by the Society for Healthcare Epidemiology of America (SHEA) [5].

In a 2015 point-prevalence study, *Clostridioides difficile* 027 was absent from healthcare institutions in Switzerland [6], although rare cases had been reported previously [7]. Within one week in December 2016, we detected three unrelated cases of patients affected by *C. difficile* 027 in our university hospital. Subsequently, an outbreak of *C. difficile* 027 occurred across several hospitals in the same network, which continued until December 2017. Here, we report on this outbreak, and how we investigated and managed it. A special focus is placed on the lean intervention measures used to halt the outbreak.

Methods

In December 2016, our central microbiology laboratory identified a potential hypervirulent *C. difficile* 027. Within four days, two other patients were found to be affected, and an outbreak investigation was started, including a detailed line list and an epidemic curve.

Case definitions

All patients from our hospital group with stool samples indicative for *C. difficile* 027 (see section on laboratory analysis) were included in this outbreak report, without any exclusion criteria, resulting in 28 patients since December 2016. Twenty patients were affected by the outbreak clone, as confirmed by WGS.

Setting

Our hospital group consists of a 950-bed tertiary care hospital, a city hospital, three regional hospitals, and a rehabilitation clinic, together caring for approximately 60,000 inpatients per year, and with a catchment area of approximately 1,000,000 inhabitants. Patients are transferred to another site within the hospital group according to their medical needs. Each hospital has its own staff, which are not shared with other sites.

Infection control measures

We noticed that initially, some of the laboratories within our hospital group only used a rapid enzyme immunoassay for the detection of *C. difficile* toxins A and B. These assays do not identify putative ribotype 027 strains. Therefore, starting the third week of the outbreak, all stool samples with a positive screening test for *C. difficile* were analyzed in the central lab using a PCR method that indicates hypervirulent strains (Figure 1).

In addition to the standard of care requiring contact isolation and a separate restroom for every patient with diarrhoea, known case patients were admitted to single rooms only. Rooms of affected patients were disinfected with a sporicidal agent (Pentapotassium

bis(peroxymonosulphate) bis(sulphate), Perform1%™, Schuelke, Hamburg, Germany) upon patient discharge. Despite these measures, additional patients tested positive, and fomites of some of those rooms were suspected to be the source of ongoing transmission. Therefore, isolation precautions and cleaning procedures were stepped up: 1) Sporicidal cleaning of rooms of *C. difficile* 027 positive patients was performed once daily; 2) Wards with more than two affected patients and therefore suspicion of transmission were at one time cleaned entirely with sporicidal agents, starting the third week of the outbreak.

Daily contact between the teams from the affected wards and the infection prevention team ensured understanding of the need for enhanced preventive measures and may have led to improved compliance with hand hygiene. The division chiefs and head nurses of the entire hospital group were notified of the outbreak and an information sheet on infection control measures for this pathogen was distributed by e-mail. In addition, clinicians were encouraged to test for *C. difficile* in any patient with new onset of diarrhoea during hospitalization, which resulted in a 31% increase of tests for *C. difficile* in the third month of the outbreak.

Thus, our lean intervention bundle consisted of three elements: 1) ensuring that patients were correctly diagnosed by harmonizing the lab approach and promoting *C. difficile* testing in all patients with diarrhoea; 2) daily ward rounds by the IPC team to raise awareness of the importance of hand hygiene using alcohol-based solutions; and 3) sporicidal environmental cleaning.

Laboratory analysis

In the tertiary care hospital of the group, stool samples are screened for *C. difficile* using glutamate dehydrogenase ELISA (GDH ELISA; C-DIFF CHEK-60 ®, Techlab, Blacksburg VA, USA), followed by real-time PCR for toxins and suspected hypervirulence (GeneXpert® *C. difficile*, Cepheid, Sunnyvale CA). A combination of positive toxin B gene, *tcdCΔ117* deletion (a

regulator gene of toxin synthesis), and positive binary toxin gene, is highly suspect of the 027 ribotype. However, in the other four hospitals of our group, stool samples were initially only tested for the presence of *Clostridioides* toxin A and B (Immunocard Toxins A&B, Meridian Bioscience Inc., Memphis TN, USA), without any further analysis. As this approach does not detect potential ribotype 027, from the third week of the outbreak on, all stool samples that screened positive for *C. difficile* were analyzed in the main microbiology laboratory using GeneXpert®. Stool samples suspected to contain *C. difficile* 027 were sent for culture, ribotyping and whole genome sequencing (MiSeq, Illumina, San Diego CA, USA) to the University Hospital Basel, starting December 2016.

PCR-ribotyping was performed using high-resolution capillary gel-based electrophoresis [8] as described elsewhere [9]. Capillary electrophoresis used the ABI-3500 Genetic Analyzer (Applied Biosystems [Life Technologies], Foster City, CA). Fragments were analysed using GeneMapper v 5.0 (Applied Biosystems) and Bionumerics v 7.6.2 (Applied Maths, Sint-Martens-Latem, Belgium) software to compare fragment profiles against the standard set of the ECDC Brazier strain collection of PCR ribotypes, obtained from the European *Clostridium difficile* infection study network (ECDIS-NET).

Whole-Genome Sequencing

All suspected 28 *C. difficile* 027 isolates underwent DNA extraction using EZ1 Advanced XL (Qiagen, Hilden, Germany), except for one sample which did not show growth. Resulting DNA was sequenced on the Illumina MiSeq (300 bp paired end reads) or NextSeq (150 bp paired end reads) platforms following Nextera XT or Nexteraflex library creation. The genome of isolate CdBe2 was assembled in CLC Genomics Workbench 9.5.3 giving 575 contigs totalling 4.2Mb. All data were mapped within CLC Genomics Workbench 12.0.3 against this reference genome

giving mean read depth over 52x in all cases but one (37x). All WGS data is available from the European Nucleotide Archive (https://www.ebi.ac.uk/ena/) under project PRJEB37809.

Ethical considerations

Given the fact that this outbreak investigation was conducted as part of the portfolio of duties by our intervention prevention unit and considered quality assurance, institutional review board approval was not required.

Results

Outbreak description

The first detection of a potential ribotype 027 in stool samples of three patients within one week triggered an outbreak investigation. Infections with the outbreak clone affected twenty patients, with a mean age of 77 years (range, 56 to 88 years); all were inpatients, and all had received antibiotics before presenting with CDI. Three patients (15%) died as a result of the infection; a fourth patient died of sepsis of unknown cause two weeks after in-patient treatment for CDI. Three patients (15%) suffered from relapses (in total, seven episodes), requiring five readmissions for colitis in two of those patients. One patient was treated with two fecal microbiome transplantations from her son, as relapses occurred despite several courses of antibiotic treatment. The subsequent length of stay was 13 days once CDI had been diagnosed (median; range 7.25 to 20 days), compared to an overall average stay of 6 days in our hospital group.

Four out of five hospitals of our hospital group were involved in the outbreak, across eleven individual wards. We noted clustering of cases, with one specific ward in hospital A witnessing six patients and another ward in hospital B having seven patients, with few patients being

transferred between hospitals. A spatio-temporal investigation revealed shared restrooms, shared rooms and care provided by the same healthcare workers as the most likely sources of transmission. Being admitted to the same ward as a CDI patient, but not the same area within that ward, for a time-period of only 20 hours proved sufficient for transmission in one case. However, for a few patients, the transmission route could not be established, *e.g.*, one patient had no other feature in common with a symptomatic patient other than having a cardiac ultrasound performed using the same equipment a few hours later. Certain other institutions use the Bern University microbiology laboratory for processing their samples; the revised algorithm allowed us to detect one further case in a regional hospital outside our network. This patient had never visited our hospital network before being diagnosed with CDI, but was transferred to one of our rehabilitation clinics afterwards.

Most cases were detected within a three-month period after the beginning of the outbreak. In our hospital network, no new infections due to this strain were identified after December 2017, and this remains the case as of July 15th, 2020 (Figure 1).

Outbreak strain characterization by WGS

In all stool samples highly suspect of ribotype 027 by GeneXpert®, this hypervirulent ribotype was confirmed by ribotyping, with the exception of one sample from which *C. difficile* could not be cultured.

Ribotyping may show limited information in terms of resolution, as outbreak and non-outbreak related isolates with the same ribotype cannot be differentiated. Therefore, we conducted an analysis using whole genome sequencing. Phylogenetic analysis of all *C. difficile* 027 isolates confirmed that all outbreak isolates (samples CdBe01-20) are very closely related, being identical across the whole genome with the exception of 1-3 SNP differences, seen in six isolates (Figure 2). Seven further ribotype 027 isolates were identified during 2017 (samples CdBe21-27), which

showed over 30 SNP differences to the outbreak strain, suggesting that these are unlikely to be direct transmissions; they also were not epidemiologically linked.

Discussion

Several hospitals in our network were affected by this outbreak caused by a single clone of *C. difficile* 027, a ribotype not identified in a nationwide point-prevalence study the year before.

In order to facilitate implementation, we opted for a lean intervention bundle to counter this outbreak: focusing on raising awareness of this hypervirulent ribotype, harmonizing the diagnostic approach, strict hand hygiene, and sporicidal cleaning.

Stool samples of two of the earliest patients had tested positive for *C. difficile* by Immunocard toxin testing three weeks before their confirmation as ribotype 027. Most likely they would have been identified as suffering from *C. difficile* 027, had adequate diagnostic methods been employed. This delayed the recognition of the outbreak and thus enabled spreading of the hypervirulent ribotype, as indicated by missing epidemiological links among some of the first patients. Standardizing the lab diagnostic procedure allowed identification of stool samples with a possible 027 strain, which was confirmed by WGS in all cases but one. Detailed phylogenetic analysis using WGS based data revealed that 20 isolates fell within three SNPs of the reference case, which is highly suggestive of transmission of the outbreak clone between individual cases.

Transmission most probably occurred through contaminated hands of healthcare workers, as few patients had direct contact among each other. In several cases, being admitted to the same unit as an infected patient, but not in the same room, even for less than 24 hours, was sufficient for transmission. Residual spores not eliminated by terminal cleaning may have been another way of transmission.

Unfortunately, we could not determine how and when this pathogen was introduced into our healthcare system. Ribotype 027 is the most common *C. difficile* ribotype reported in European countries besides Switzerland [10]. However, the standard screening of repatriated patients arriving in our hospital currently does not include *C. difficile*, so we have insufficient insight into transmission dynamics.

In order not to undermine adherence to our modified contact precautions (which does not require gloves or gowning unless if anticipating contact with bodily fluids [11]), we did not require glove use for every contact with a CDI patient, nor did we enforce hand washing with soap and water instead of our alcoholic handrub. This decision was taken despite the fact that handrub alcohol does not kill *C. difficile* spores.

According to the 2018 IDSA clinical practice guidelines for *C. difficile* infection, in endemic settings, either soap and water or an alcohol-based hand hygiene product can be used (strong recommendation, moderate quality of evidence), whereas in outbreaks, hand hygiene with soap and water should be given preference (weak recommendation, low quality of evidence) [12]. Likewise, the European Society of Clinical Microbiology and Infectious Diseases Study Group for *C. difficile* recommends switching from alcohol-based handrub to hand washing in outbreak settings (conditional recommendation, very low quality of evidence), as well as using gloves and gowns (strong recommendation, very low quality of evidence) [13].

Despite these recommendations, we felt that there was no need for stepping up and propagating general glove use or hand washing with water and soap prior to leaving the patient room, as the installed bundle halted the outbreak.

Daily sporicidal cleaning of affected patients' rooms and one-time sporicidal cleaning of entire wards with possible transmission proved to be sufficient to substantially reduce hospital-acquired CDI, as described in one other report [14].

Other reported *C. difficile* 027 outbreaks were controlled with: terminal cleaning [15] or cleaning of an entire facility [16] including adjacent rooms upon discharge of a CDI patient [17]; efficient case identification and treatment [18-20]; isolating CDI patients in single rooms [19, 20] or on a dedicated ward [18, 21]; isolating patients with diarrhoea until *C. difficile* was ruled out [21]; and restricting fluoroquinolone use [18-21]. Some reports describe the successful use of hydrogen peroxide for environmental disinfection [19], also as a vaporized preparation [17, 20], or chlorine-containing disinfectants [20, 21]. In contrast, daily cleaning of a CDI patient's room and of the bedpan cleaning area with non-sporicidal disinfectants (chloride concentration < 1000 p.p.m.) actually *increased* CDI incidence in one report [22]. Selective decontamination of the digestive tract in ICU patients (using oropharyngeal and intestinal applications of colistin, tobramycin and amphotericin in combination with systemic cefotaxime during the first two to four days) during an outbreak also increased CDI risk [19]. Information campaigns to medical personnel were key in several reports [16, 20, 21, 23]. Most reports, however, stressed reinforcing hand hygiene [19-21], some also by the affected patients themselves [18, 20], and wearing gloves and gowns [19, 20].

To our knowledge, so far no outbreak has been managed by continuing the usual hand hygiene with alcoholic solutions and by explicitly refraining from both handwashing with soap and water as well as default gloving and gowning. Our approach was to facilitate compliance with hand hygiene by maintaining the usual hand hygiene using alcoholic handrub, as studies suggest that this approach ensues higher compliance compared with hand washing with soap and water [24]. Alcoholic handrub can be made more easily available and its application is less time-consuming. Potential surface contamination with spores was addressed by sporicidal environmental cleaning. This is what we decided to label as "lean intervention bundle", as it was a minimalistic outbreak management strategy that resorted to few but highly effective measures. Further, given the low

level of fluoroquinolone utilization in our inpatient setting, we opted against including an antibiotic stewardship intervention in the bundle of measures to contain this outbreak.

As for the time being, not all stool samples positive for *C. difficile* are tested for ribotype 027 in our country, and because CDI is not a notifiable disease in Switzerland, individual cases may be missed and the spread of potentially hypervirulent strains underestimated. Therefore, we recommend establishing a nationwide screening for hypervirulent ribotypes of all *C. difficile* positive stool samples, as well as mandatory notification of health authorities. In case of clustering of *C. difficile* cases, WGS is to be employed to check for clonality.

Limitations of our study include the fact that, because of possible lack of clinical vigilance, and due to the previous absence of testing for ribotype 027 in peripheral hospitals of our network, related cases prior to the identified index case may have been missed.

Conclusion

In conclusion, this *C. difficile* 027 outbreak was caused by a single strain with an unknown source. Ribotyping alone did not allow strains to be recognized as outbreak clones; this resolution was achieved by WGS only. The response to this outbreak without gloving and gowning or using soap and water for hand hygiene, but with sporicidal cleaning, proved to be efficient, and suggests that such lean intervention bundles may save resources while achieving their goals.

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Other authors: None.

Conflict of interest

None.

Disclosure

All authors have approved the final article.

Authors' contribution

Outbreak investigation and management: SB, AK, JM, NB

Laboratory method harmonization: CC, RS

Whole genome sequencing and analysis: HSS, AE

Writing of manuscript: AK, JM

Critical reviewing of the manuscript: all authors.

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None

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Figure legends

Figure 1

Epidemiological curve of 027 isolates and interventions

Figure 2

Phylogeny of *C. difficile* 027 isolates from this study.

This neighbour joining single nucleotide polymorphism (SNP) phylogeny used the assembly of isolate CdBe02 as a reference (shown in bold), rooted using unrelated 027 isolates. It was generated in CLC Genomics Workbench 12.0.3 with parameters that differed from the default as: variant calling with 10x minimum coverage, 10 minimum count and 70% minimum frequency, and SNP tree creation with 10x minimum coverage, 10% minimum coverage, 0 prune distance and including multi-nucleotide variants (MNVs). Outbreak isolates show a diversity of up to three SNPs from the reference. Specific examples of epidemiological links between outbreak isolates are superimposed.

Figure S1

Phylogeny of *C. difficile* 027 isolates including the external laboratory samples. This phylogeny used the assembly of isolate CdBe02 as a reference (shown in bold), rooted using unrelated 027 isolates. Outbreak isolates show a diversity of up to six SNPs from the reference.

417 **Figure 1**

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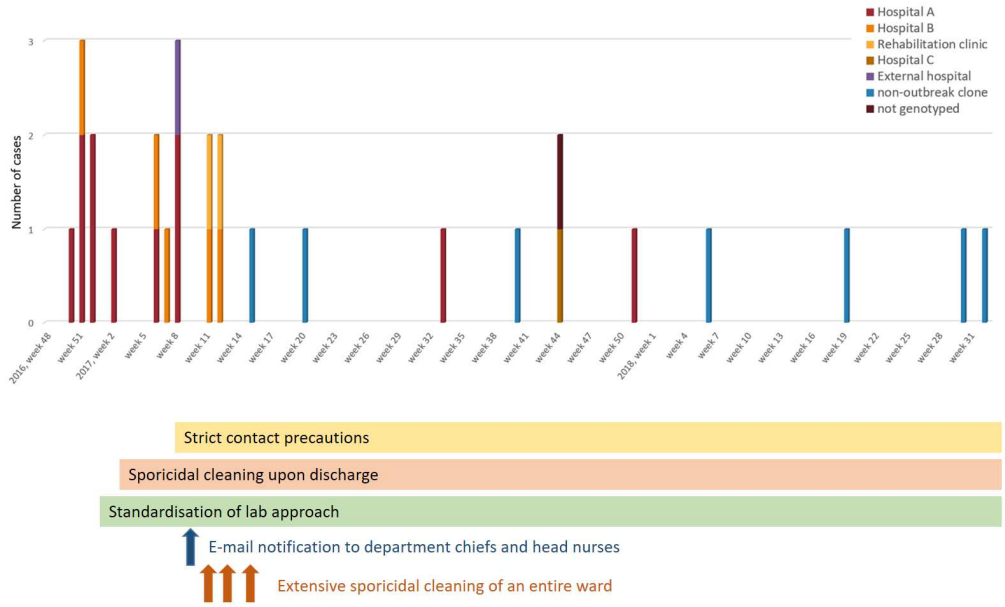

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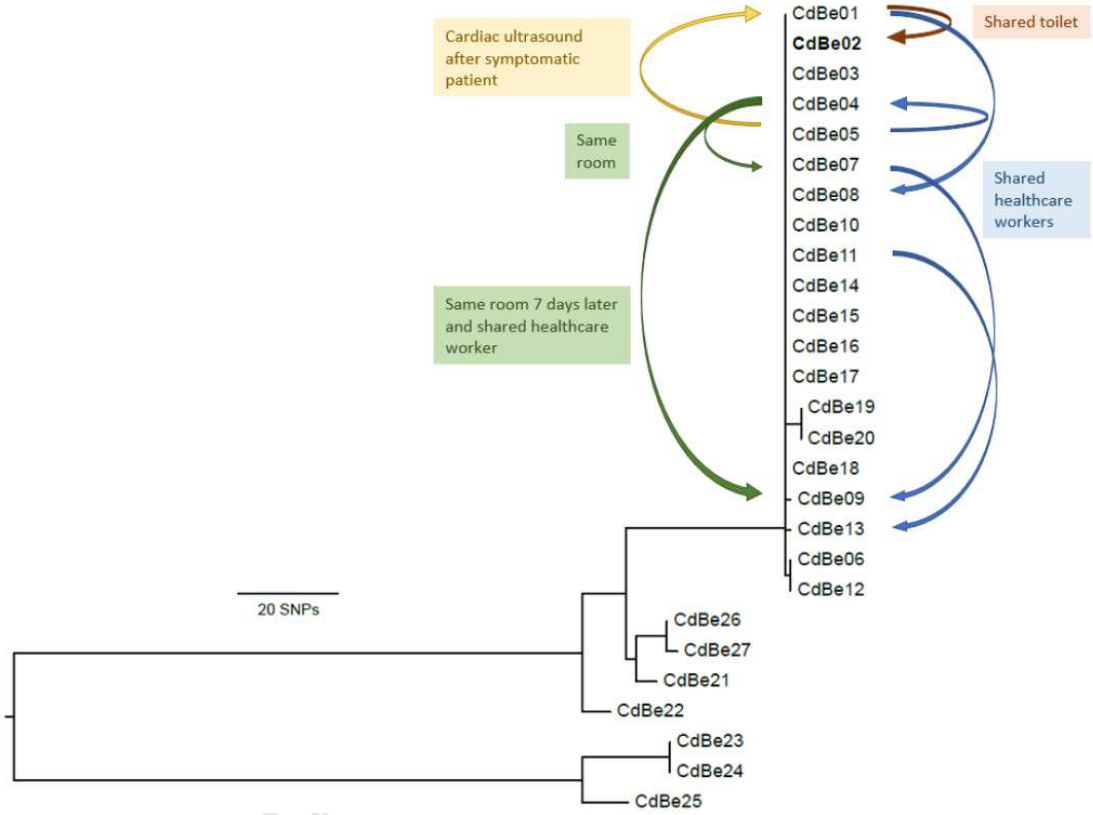
423 **Figure 2**



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Supplement

In order to better understand the outbreak, we collaborated with a private laboratory, labormedizinisches zentrum Dr. Risch, serving private hospitals and practices in our region. In this laboratory, stools are screened for the presence of toxigenic *Clostridoides difficile* using the algorithm proposed by Fenner *et al*. [25]. Reactive screening tests are confirmed by PCR (GeneXpert CDIF®). In case of suspected ribotype 027, the stool specimens are sent to an expert laboratory for confirmation (AE) using ribotyping and whole genome sequencing. In hospitalized patients, positive test results prompt timely, automated alerts to the sender as well as the respective hospital hygiene teams.

Stool samples analysed at the external private laboratory revealed 14 further patients (median age 82.5 years, range 53 to 93 years) belonging to this cluster. As these outpatients' charts could not be accessed, we were unable to analyze the outpatients' outcomes and epidemiological links outside of our hospital group.

Only two of these patients had been hospitalized in our hospital group: one patient was admitted six days after a symptomatic patient into an adjacent ward, the second was managed on the same ward as another of our patients five months earlier, making a transmission on that ward rather unlikely. Of these, one patient had diarrhoea after receiving antibiotic therapy as an inpatient, but was tested for *C. difficile* only after discharge two weeks later; the second patient was diagnosed with CDI nine months later. Two other outpatients had been seen at our cardiology outpatient clinic in late 2016 two and seventeen days after a symptomatic inpatient of this cluster did, respectively. Of note, the ultrasound examinations were performed by different physicians, so possible transmissions are suspected to have occurred via fomites. However, these outpatients were diagnosed with the outbreak strain 14 months and 17 months after the clinic visit, so an epidemiological link is uncertain.

450 Samples from the external laboratory (CdRi01-12) are shown in Figure S1.

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453 **Figure S1**

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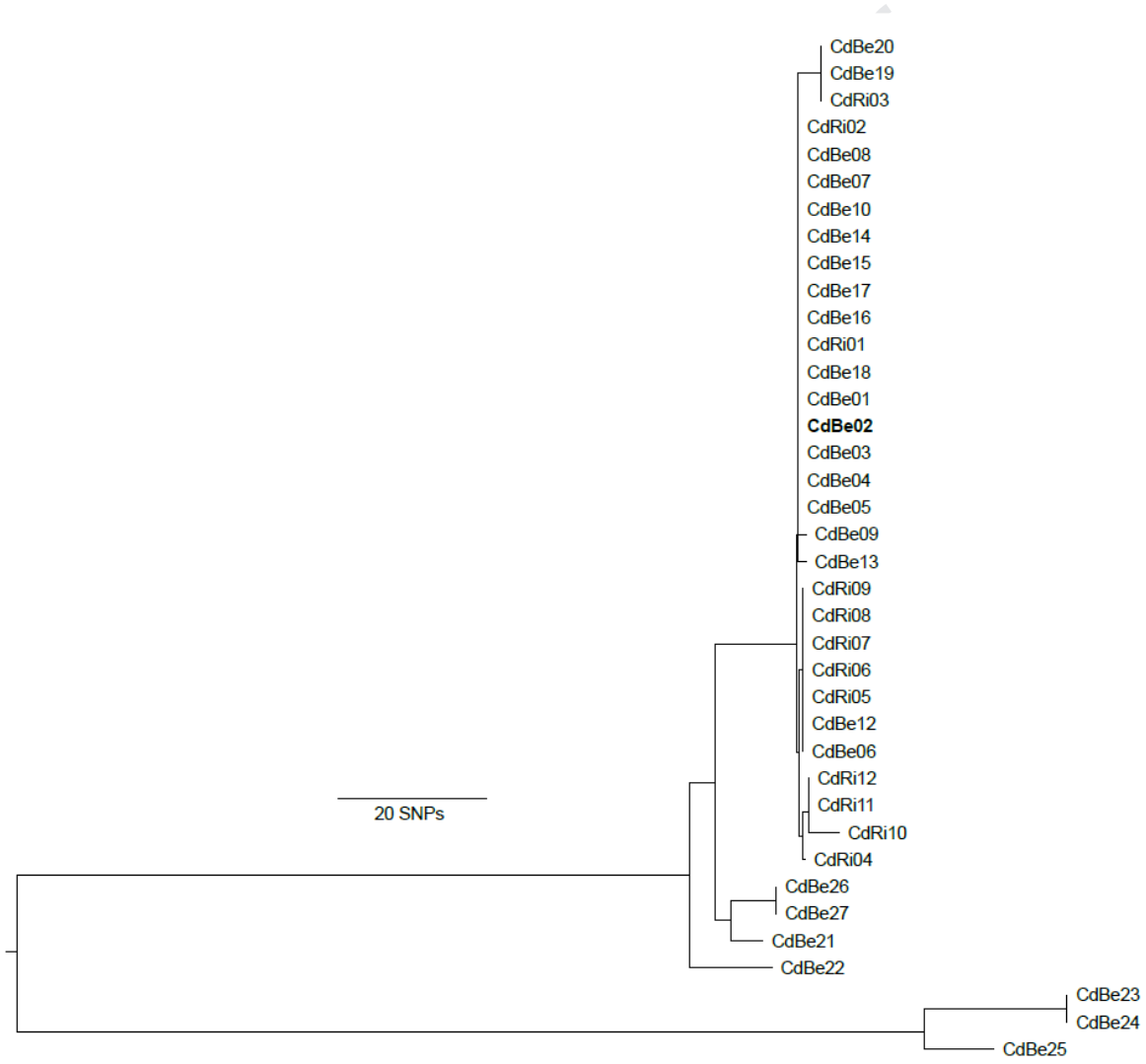

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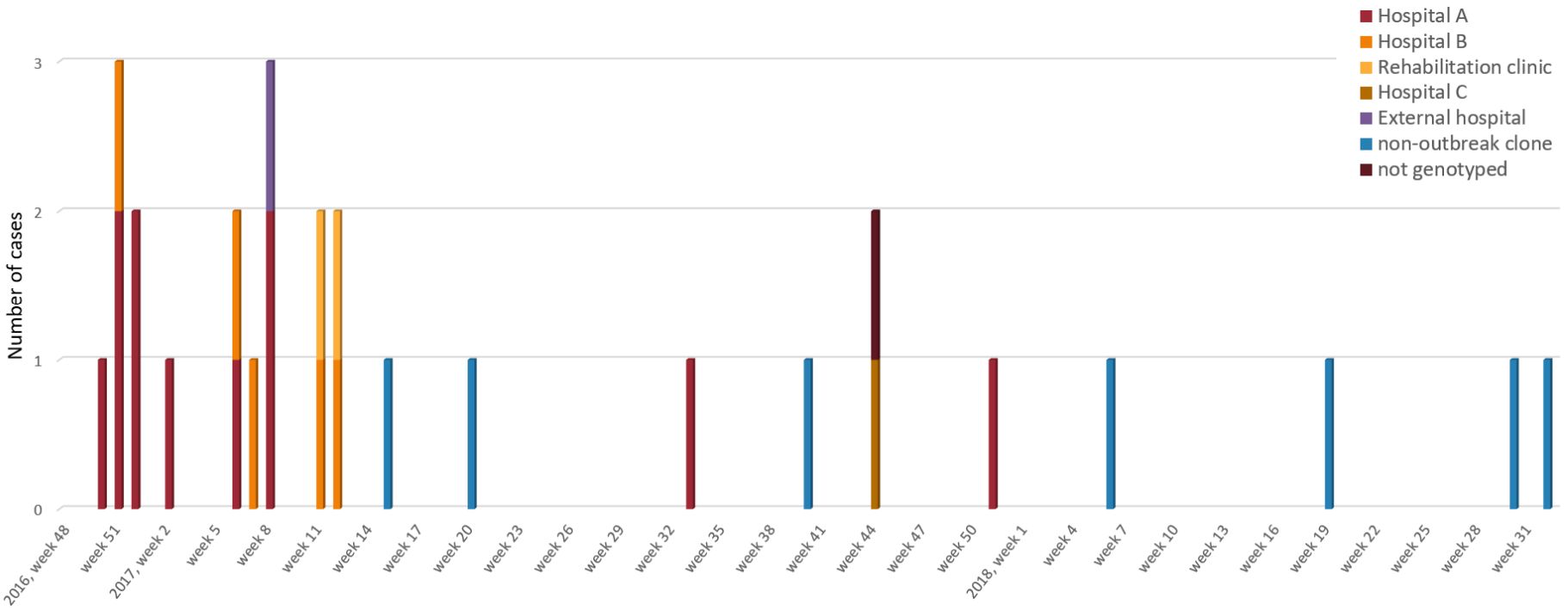


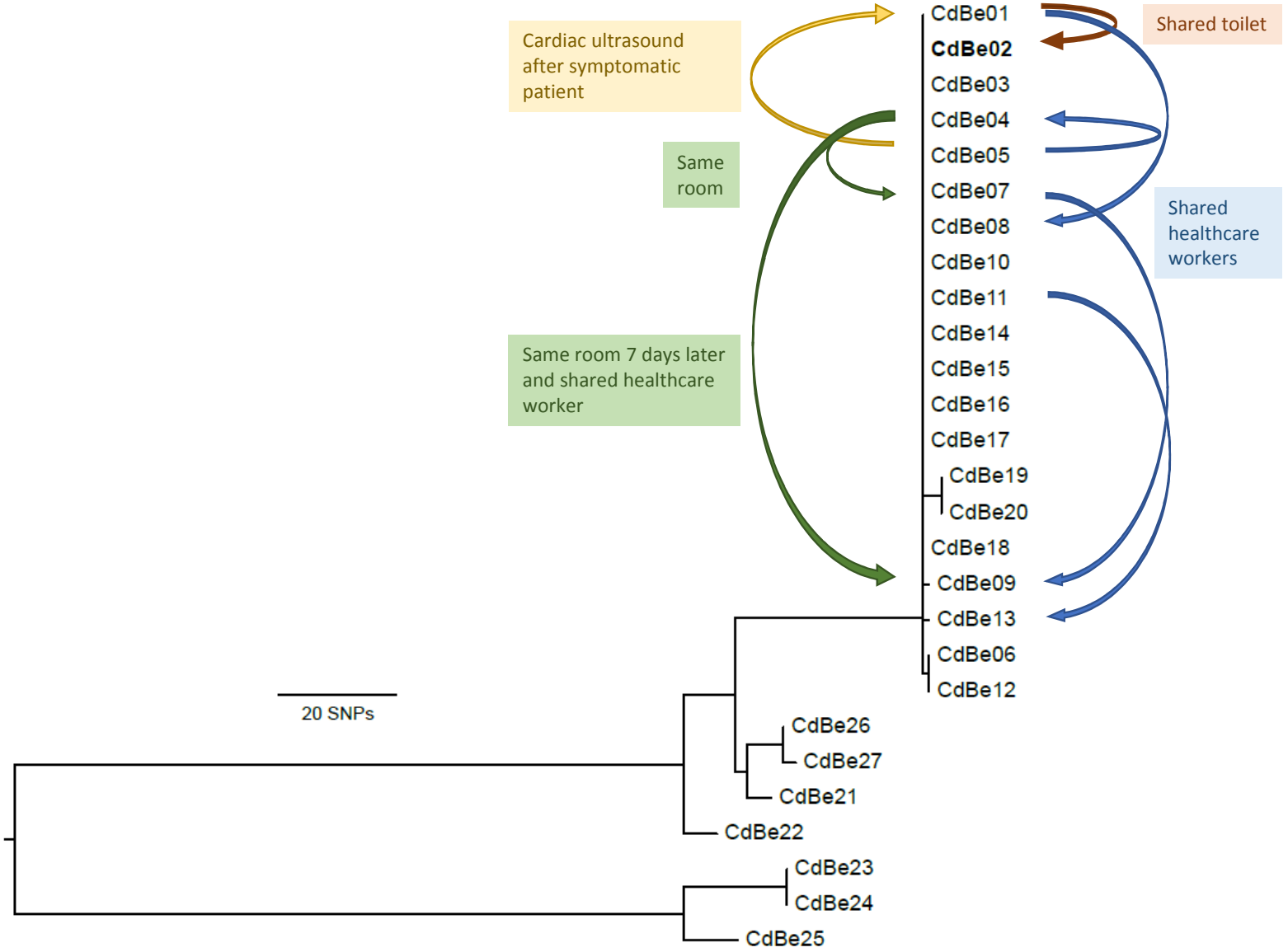
Strict contact precautions

Sporicidal cleaning upon discharge

Standardisation of lab approach

E-mail notification to department chiefs and head nurses

Extensive sporicidal cleaning of an entire ward


Cardiac ultrasound after symptomatic patient
Same room
Same room 7 days later and shared healthcare worker
Shared toilet
Shared healthcare workers
CdBe01
CdBe02
CdBe03
CdBe04
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